International Preliminary Examining Authority
European Patent Office
Directorate General 2
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GERMANY

6 January 2006

Sent by fax

Dear Sirs

International Patent Application No. PCT/GB2004/005462
DELTA BIOTECHNOLOGY LIMITED
Our ref: DELBE/P32303PC

This is a response to the Written Opinion of the International Preliminary Examining Authority (the "IPEA") dated 7 November 2005.

Amendment

We enclose replacement page 131 to replace page 131 on file.

Part (b) of Claim 1 is amended to refer to "co-expression" rather than "expression". Basis for this amendment can be found, *inter alia*, on page 5, line 10.

Novelty

The IPEA alleges that Claim 1 lacks novelty over D1, asserting that D1 inherently discloses expression of chaperones as GST fusions. If chaperones were expressed in D1, which is by no means certain, they were expressed from a single open reading frame comprising a GST coding sequence and a chaperone coding sequence i.e. as a single fusion protein.

Claim 1, as amended, refers to "*co-expression of the gene encoding protein comprising the sequence of the chaperone protein and the gene encoding the non-2μm-family plasmid protein* (emphasis added)". The term co-expression applies to the expression, at the same time, of two or more genes encoded by different open reading frames. Therefore, Claim 1 as amended clearly does not encompass the subject matter that the IPEA considers to be inherently disclosed by D1. Accordingly, Claim 1 is novel over D1.

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Inventive Step

With respect, we submit that the preliminary view taken on inventive step in the Written Opinion of the ISA overlooked much of the relevant prior art in the field. When the present invention is considered more fully in the context of the teachings of all relevant documents in the art, as explained in more detail below, it is readily apparent that the claims are inventive.

As discussed above, the present invention relates to expression systems for improving the production of heterologous proteins, and involves the co-expression of genes for a heterologous protein and a chaperone *from the same 2μm plasmid.*

The prior art had taught that heterologous protein production could be improved by co-expression with a chaperone such as protein disulphide isomerase ("PDI"), but that the transgenes for the chaperone and the heterologous protein should be chromosomally integrated. Not only did the prior art favour chromosomal integration, but it also *actively discouraged* the expression of these transgenes from multi-copy vectors such as the 2μm plasmid.

The person skilled in the art is taught, by the prior art, that heterologous protein production can be improved by simultaneous over-expression of a chaperone, but that the chaperone gene should be chromosomally integrated, and that the use of a 2μm plasmid to express a chaperone should be avoided:

- Robinson *et al*, 1994, *Bio/Technology*, **12**, 381-384 (cited in the specification on page 4, lines 4-8; copy enclosed) reports that a chromosomally integrated copy of a gene for PDI could be used to increase the expression of heterologous proteins in a yeast host cell. Robinson *et al* goes on to report (see page 383, third paragraph of the "*Discussion*") that –

  > "*An alternative to stable single copy expression of PDI is to employ a multicopy 2μ expression vector to further increase PDI levels. However, we have actually observed detrimental effects on heterologous protein secretion resulting from multicopy overexpression of PDI*".

  There can be little doubt that Robinson *et al* discourages the person skilled in the art from attempting to increase heterologous protein production by co-expression with a chaperone (such as PDI) from a multicopy vector, such as the 2μm vector.

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- Bao *et al*, 2000, *Yeast*, **16**, 329-341 (cited in the specification on page 4, line 30; copy enclosed) similarly reports that over-expression of the *Kluyveromyces lactis* PDI gene ("*KlPDI1*") is toxic when expressed *from a multicopy plasmid*. By contrast, duplication of the *KlPDI1* gene *in the chromosome was well tolerated* and led to an increase in heterologous protein production. This is discussed in the section entitled "*Overexpression of KlPDI1 gene is toxic to K. lactis cells*", which starts on page 333 and ends on page 336. Accordingly, the reader of Bao *et al* would be motivated to avoid expression of a chaperone (such as PDI) from a multicopy vector (such as the 2μm plasmid), and certainly would not be inclined to attempt to maximise heterologous protein production by co-expression of a chaperone and a heterologous protein from the same 2μm plasmid. In light of Bao *et al* the person skilled in the art would be most surprised to find that the system of the present invention is very effective at increasing heterologous protein production.

- Additionally, D4 reports that protein secretion was increased "*by duplication of either KlPDI1 or KlUBI4 in the host chromosome*" (D4, page 103, final sentence; emphasis added). D4 goes on to explain that –

  > *"To increase the gene dosage of KlPDI1 in the rHSA producing yeast, it was first thought that the KlPDI1 gene might be directly introduced into the multi-copy vector that carried the rHSA expression cassette. However, such constructs were found to severely affect yeast growth and plasmid stability ... Therefore, we have chosen the strategy of introducing a single duplication of either the KlPDI1 or KlUBI4 gene on the host chromosome"* (page 105, section 3-1, first paragraph).

  There can be no clearer teaching to the person skilled in the art that one should avoid co-expression of a heterologous protein with a chaperone (such as PDI) from the same 2μm plasmid.

Furthermore, the person skilled in the art is taught that, in order to maximise heterologous protein production by simultaneous over-expression of a chaperone, the gene encoding the heterologous protein should be chromosomally integrated, in preference to presenting the gene for the heterologous protein on a multicopy vector:

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- D3 reports that it is preferable for a recombinant gene encoding a heterologous protein, in this case bovine pancreatic trypsin inhibitor ("BPTI") to be chromosomally integrated (using a δ integration vector), rather than expressed from a multicopy vector such as the 2μm plasmid. Specifically, D3 reports that –

  > "*The relationship between expression level and secretion of...BPTI...was determined in Saccharomyces cerevisiae using a tunable amplifiable δ integration vector. Optimal secretory productivity...yields...an order of magnitude increase over 2μ plasmid-directed secretion*" (Abstract, first and second sentences; emphasis added).

In light of the above discussed prior art, it is clear that not only is there no motivation in the art to attempt to optimise heterologous protein production by co-expression of genes for a heterologous protein and a chaperone from the same 2μm plasmid, but additionally the art actively discourages the person skilled in the art from attempting to do so.

Consequently, the person skilled in the art would be most surprised to find that the expression system of the present invention has significant advantages, in terms of heterologous protein production, compared to expression systems which utilise chromosomally integrated transgenes.

For example, please see Example 4 of the present application, which assesses the production of recombinant human transferrin ("rTf") encoded by a gene on a 2μm plasmid, in a strain of yeast comprising an additional chromosomal copy of PDI ("Strain A") compared to a yeast strain in which an additional copy of PDI is provided on the same 2μm plasmid as the rTf gene (the plasmid encoding both PDI and rTf is designated pDB2711).

In light of the teachings of the prior art, as discussed above, the person skilled in the art would expect that plasmid pDB2711 would be detrimental to the growth of the yeast and its productivity of rTf, and certainly less productive than Strain A. However, surprisingly, the applicants show that the converse is true. Table 3 on page 96 shows that the level of rTf production is approximately at least 5-fold higher in the yeast strain carrying PDI on the 2μm plasmid, compared to the strain carrying an additional chromosomal copy of PDI. This was a most surprising finding, which could not have been predicted from the prior art.

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Accordingly, it is clear that claims of the present application are based on an inventive development in the art.

The IPEA questions whether the claims of the present application are inventive insofar as they encompass the co-expression of a cytosolic protein and a chaperone from the same 2μm plasmid. However, as explained in detail above, it is clearly inventive to express a chaperone from a 2μm plasmid, and this is not changed by co-expression of a protein product from the same plasmid, whether that protein product is secreted or cytoplasmic. Therefore the claims can be clearly seen to be inventive.

The IPEA have also alleged that D1 shows expression on a genome-wide basis of proteins from a 2μm-family plasmid without any apparent technical difficulty. The IPEA maintains that D1 is relevant to the issue of the prejudice in the art exemplified by D3 and D4 and the other documents discussed above.

In particular, the International Examiner has commented that "quantitative" yields of the fusion proteins would have been obtained in D1. However, this does not make D1 a document that the person skilled in the art would naturally have looked to for guidance in optimising the production of a heterologous protein product. On the contrary, according to D1, GST-ORFs from individual yeast strains were assayed for biochemical activities, which were identified as being either present or absent (page 1153, fourth paragraph), and therefore D1 merely describes a qualitative method.

To overturn the prejudice in the art against expressing heterologous proteins and chaperones from multi-copy plasmids for the purpose of maximising the productivity of the expression system (e.g. as in D3, D4, Robinson *et al*, and Bao *et al*, as discussed above), D1 would have to show that expression of a chaperone from a multicopy plasmid permitted increased co-expression of a heterologous protein. Even if one accepts that the teaching of D1 inherently discloses that chaperones were expressed from a 2μm plasmid, which is by no means clear, there is no evidence that this expression had a quantitative effect on the co-expression of a heterologous protein. In fact, D1 does not even describe co-expression of a heterlogous protein and a chaperone, only the expression of a single GST-ORF fusion protein. Accordingly, D1 says nothing to a person skilled in the art seeking to maximise the effect of co-expression of a chaperone with a heterologous gene about the benefits or disadvantages of choosing to express the chaperone and/or the heterologous protein from a 2μm plasmid.

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The IPEA has also commented that the claims do not specify a time frame for the protein expression step, so as to conclude that the issue of the time frame is therefore irrelevant to the issues in dispute. With respect, we disagree. We previously argued that the disclosure of D1 would have been considered, by the skilled person, to not be relevant to the teachings of D4 and D3 because the period during which proteins were expressed in D1 was so short that effects on cell viability would not become apparent. The problem apparent in the art, from D3 and D4, was that high level expression of a chaperone or other heterologous protein from a multi-copy plasmid resulted in a deleterious effect on cell viability and expression of heterologous protein. D1 would do nothing to dissuade the skilled person of this fact.

From the foregoing comments it can be seen that D1 fails to provide any motivation to deviate from the teachings of D4 and D3. Accordingly, the claims possess an inventive step in the light of the cited prior art.

Yours faithfully

Richard Bassett
For and on behalf of Eric Potter Clarkson LLP

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Encs: Replacement page 131
Robinson *et al*, 1994
Bao *et al*, 2000